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(54) Title: METHOD TO DIAGNOSE HEREDITARY HEMOCHROMATOSIS

(57) Abstract

New genetic markers for the presence of a mutation in the common hereditary hemochromatosis (HH) gene are disclosed. The multiplicity of markers permits definition of genotypes characteristic of carriers and homozygotes containing this mutation in their genomic DNA.

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METHOD TO DIAGNOSE HEREDITARY HEMOCHROMATOSIS

Technical Field

The invention relates to genetic tests for subjects carrying one or two copies of a mutated gene associated with hereditary hemochromatosis. More specifically, the invention concerns utilization of new markers associated with a common mutation in this gene which indicate the presence or absence of the mutation.

Background Art

Hereditary hemochromatosis (HH) is an inherited disorder of iron metabolism wherein the body accumulates excess iron. In symptomatic individuals, this excess iron leads to deleterious effects by being deposited in a variety of organs leading to their failure, and resulting in cirrhosis, diabetes, sterility, and other serious illnesses. Neither the precise physiological mechanism of iron overaccumulation nor the gene which is defective in this disease is known.

HH is inherited as a recessive trait; heterozygotes are asymptomatic and only homozygotes are affected by the disease. It is estimated that approximately 10% of individuals of Western European descent carry an HH gene mutation and that there are about one million homozygotes in the United States. Although ultimately HH produces debilitating symptoms, the majority of homozygotes have not been diagnosed. Indeed, it has been estimated that no more than 10,000 people in the United States have been diagnosed with this condition. The symptoms are often confused with those of other conditions, and the severe effects of the disease often do not appear immediately. It would be desirable to provide a method to identify persons who are ultimately destined to become symptomatic in order to intervene in time to prevent excessive tissue damage. One reason for the lack of early diagnosis is the inadequacy of presently available diagnostic methods to ascertain which individuals are at risk.

Although blood iron parameters can be used as a screening tool, a confirmed diagnosis often employs HLA typing, which is tedious, nonspecific, and expensive and/or liver biopsy which is undesirably invasive and costly. Accordingly, others have attempted to develop inexpensive and noninvasive diagnostics both for detection of homozygotes having existing disease, in that presymptomatic detection would guide intervention to prevent organ damage, and for identification of carriers. The need for such diagnostics is documented for example, in Finch, C.A. West J Med (1990) 153:323-325; McCusick, V. *et al.* Mendelian Inheritance in Man 11th ed., Johns Hopkins University Press (Baltimore, 1994) pp. 1882-1887; Report of the Joint World Health Organization/HH Foundation/French HH Association Meeting, 1993.

Although the gene carrying the mutation associated with HH is at present unknown, genetic linkage studies in HH families have shown that the gene responsible in Caucasians resides on chromosome 6 near the HLA region at 6p2.13 (Cartwright, Trans Assoc Am Phys (1978) 91:273-281; Lipinski, M. *et al.* Tissue Antigens (1978) 11:471-474). Within this gene a single mutation gives rise to the majority of disease-causing chromosomes present in the population today. This is referred to herein as the "common" or "ancestral" or "common ancestral" mutation. These terms are used interchangeably. It appears that 80-90% of all HH patients carry at least one copy of a common ancestral mutation which carries with it specific forms of certain markers close to this ancestral HH gene. These markers are, as a first approximation, in the allelic form in which they were present at the time the HH mutation occurred. See, for example, Simon, M. *et al.* Am J Hum Genet (1987) 41:89-105; Jazwinska, E.C. *et al.* Am J Hum Genet (1993) 53:242-257; Jazwinska, E.C. *et al.* Am J Hum Genet (1995) 56:428-433; Worwood, M. *et al.* Brit J Hematol (1994) 86:833-846; Summers, K.M. *et al.* Am J Hum Genet (1989) 45:41-48.

Although each of such markers is putatively useful in identifying individuals carrying this defective HH gene, of course, crossing over events have, over time, separated some of the ancestral alleles from the relevant genetic locus

that is responsible for HH. Therefore, no single marker is currently specific enough to identify all individuals carrying the ancestral HH mutation.

Several markers at the approximate location of the gene associated with HH have been described. Gyapay, G. *et al.* Nature Genetics (1994) 7:246-339 describe the markers D6S306 and D6S258 which have been demonstrated hereinbelow to be in the immediate region of the HH gene. These markers consist of microsatellite regions containing $(CA)_n$ repeats of various lengths. Worwood, M. *et al.* Brit J Hematol (1994) 86:833-846 (*supra*) describes an allele at D6S265 and Jazwinska, E.C. *et al.* Am J Hum Genet (1993) 53:242-257 (*supra*) describes D6S105 as associated with an HH-specific genotype. Stone, C. *et al.* Hum Molec Genet (1994) 3:2043-2046 describes an additional HH-associated allele at D6S1001. As described hereinbelow, a multiplicity of previously undiscovered microsatellite markers and the relevant allele associated with the ancestral HH gene defect have now been found permitting the detection of genotypes with very high probabilities of being associated with the presence of the common HH mutated gene. In addition, 3 single base-pair polymorphisms associated with the HH gene have been identified, which can be included in additional diagnostic genotypes. The diagnostic genotypes described below as associated with HH are rare in the general population, consistent with the frequency of the HH gene mutation. However, they are present in a large majority of individuals affected by HH. Accordingly, the presence or absence of these genotypes can be used as a rapid, inexpensive and noninvasive method to assess an individual for the presence or absence of the common version of the defective HH gene.

Disclosure of the Invention

The invention is directed to a convenient method to assess individuals for the presence or absence, or the likelihood of said presence or absence, of a common HH-associated mutation using genetic techniques that are readily applied

noninvasively. Only a sample containing the subject's cells containing genomic DNA from the subject to be tested is required. The present invention includes materials and kits useful in conducting the genetic tests. The allelic variants at specific locations close to the HH gene are marked by distinctive lengths of microsatellite repeats or by specific single base-pair differences in DNA sequence (referred to herein as a "base-pair polymorphism").

Thus, in one aspect, the invention is directed to a method to determine the likelihood of the presence or absence of a hereditary hemochromatosis (HH) gene mutation in an individual, which method comprises obtaining genomic DNA from the cells of said individual and assessing said DNA for the presence or absence of a genotype defined by at least one nonoptional marker comprising the following microsatellite repeat alleles: 19D9:205; 18B4:235; 1A2:239; 1E4:271; 24E2:245; 2B8:206; 3321-1:197; 4073-1:182; 4440-1:180; 4440-2:139; 731-1:177; 5091-1:148; 3216-1:221, 4072-2:148; 950-1:142; 950-2:164; 950-3:165; 950-4:128; 950-5:180; 950-6:151; 950-8:137; and 63-1:151. In the notation employed for the microsatellite repeat alleles, the number subsequent to the colon indicates the number of nucleotides in the HH-associated allele between and including the flanking primers when the primers are those illustrated herein. The absence of this genotype indicates the likelihood of the absence of the HH gene mutation in the genome of said individual. The presence of this genotype indicates the likelihood of the presence of this HH gene mutation in the genome of said individual.

While the presence of only one of these alleles indicates an increased likelihood for the presence of the common ancestral genetic HH defect, the likelihood is further enhanced by the presence of multiple alleles among these nonoptional markers. Thus, the genotypes to be determined preferably include at least two, more preferably at least three, and more preferably still, at least four, preferably more than four, of these alleles. In addition, the statistical certainty of the results is enhanced by combining the information concerning the presence or absence of one or more of these nonoptional alleles with the information

concerning the presence or absence of diagnostic alleles known in the art, including D6S258:199, D6S265:122, D6S105:124, D6S306:238, D6S464:206; and D6S1001:180. The predictive power of such disease-associated alleles when measured in human genomic DNA can be quantified. An example of a computerized method for this is given in Terwilliger, J.D. Am J Hum Genet (1995) 56:777-787.

In addition, HHP-1, HHP-19, and HHP-29 (described below) base-pair polymorphisms have been established; the presence of the HH-associated allele of one of these base-pair polymorphisms especially in combination with any HH-mutation-associated microsatellite repeat allele indicates the presence of the common HH mutant gene.

Thus, in another aspect, the invention is directed to a method to determine the presence or absence of the common hereditary hemochromatosis (HH) gene mutation in an individual, which method comprises obtaining genomic DNA from the individual; and assessing the DNA for the presence or absence of the HH-associated allele of the base-pair polymorphism designated herein at HHP-1, HHP-19, or HHP-29; wherein the absence of the HH-associated allele indicates the likelihood of the absence of the ancestral HH gene mutation in the genome of the individual and the presence of the HH-associated allele indicates the likelihood of the presence of the HH gene mutation in the genome of the individual. Preferably, the method also includes determining a genotype which is a combination of the base-pair allele with an HH-associated microsatellite repeat allele.

The invention is further directed to DNA primer pairs for PCR amplification that flank the microsatellite repeat alleles and that flank the base-pair polymorphism markers useful in the method of the invention and to kits containing these primer pairs. The invention is also directed to primers permitting determination of base-pair polymorphisms by oligonucleotide ligation assay (OLA) or by alternative methods. The invention is also directed to use of the nucleotide sequence information around the microsatellite repeats to design additional primer

pairs for amplification. Applicants have provided extensive sequence information approximately 500 bp in either direction of the markers 18B4, 19D9, 1A2, 1E4, 24E2, 2B8 and 63-1. The availability of this sequence information provides additional opportunities for the design of primers for amplification of the relevant portion of DNA.

Accordingly, the invention is also directed to primers designed on the basis of this sequence information and to a computer-readable medium having recorded thereon the nucleotide sequences set forth in Figure 1A-1W described below or fragments thereof. The claimed fragments are those that do not coincide with nucleotide sequences presently available in computer-readable form.

Brief Descriptions of the Drawings

Figure 1 shows sequence information concerning the portions of the genome surrounding several markers of the invention. Figure 1A shows 1260 bp around 18B4; Figure 1B shows 1260 bp around 19D9; Figure 1C shows 1 kb around 1A2; Figure 1D shows 1380 bp around 1E4; Figure 1E shows 1260 bp around 24E2; Figure 1F shows approximately 1 kb around 2B8; Figure 1G shows sequences bracketing 731-1; Figure 1H shows sequences bracketing 5091-1; Figure 1I shows sequences bracketing 4440-1; Figure 1J shows sequences bracketing 4440-2; Figure 1K shows sequences bracketing 4073-1; Figure 1L shows sequences bracketing 3321-1; Figure 1M shows sequences bracketing 3216-1; Figure 1N shows sequences bracketing 4072-2; Figure 1O shows sequences bracketing 950-1; Figure 1P shows sequences bracketing 950-2; Figure 1R shows sequences bracketing 950-3; Figure 1S shows sequences bracketing 950-4; Figure 1T shows sequences bracketing 950-5; Figure 1U shows sequences bracketing 950-6; Figure 1V shows sequences bracketing 950-8; Figure 1W shows sequences bracketing 63-1; Figure 1X shows sequences bracketing 65-1; Figure 1Y shows sequences bracketing 65-2; Figure 1Z shows sequences bracketing 63-2; Figure 1AA shows sequences bracketing 63-3; Figure 1BB

shows sequences bracketing 373-8; Figure 1CC shows sequences bracketing 373-29; Figure 1DD shows sequences bracketing 68-1; Figure 1EE shows sequences bracketing 241-6; Figure 1FF shows sequences bracketing 241-29.

The location of the microsatellite repeated sequence itself is underlined in these figures.

Figure 2 shows the primers used for amplification and OLA determination of the base-pair polymorphisms of the invention.

Modes of Carrying Out the Invention

A multiplicity of new markers which are of variant length microsatellite repeats associated with the ancestral mutation in the gene associated with hereditary hemochromatosis have been found and the allelic forms associated with the HH genetic defect have been characterized. In general, the markers reside on chromosome 6 in the neighborhood of the locus which is associated with the defective genotype and exhibit a multiplicity of allelic variations characterized by a variation in the number of nucleotides present in the intervening sequence between flanking sequences conserved in all subjects. The intervening nucleotide sequences consist essentially of di-, tri- and tetranucleotide repeats, most commonly the dinucleotide $(CA)_n$. As is generally known in the art, this type of repeat is known as a "microsatellite" repeat. The microsatellite repeat regions which characterize the markers of the present invention may be simple microsatellite repeats containing only one type of repeated sequence or may be compound. In addition to $(CA)_n$, $(CT)_n$ and other repeated sequences are found. These repeat sequences generically, are designated "microsatellite repeats" herein. As shown hereinbelow, the flanking sequences conserved with respect to each marker are interrupted by intervening nucleotide sequences ranging in number from about 110 to about 300 bases. Generally, the size of each allele differs within the context of a single marker by 2-4 bases from the next closest allele.

As used herein, "marker" refers either to a base-pair polymorphism or to a microsatellite region wherein varying numbers of $(CA)_n$ or other microsatellite repeats are flanked by conserved regions; advantage can be taken of the conserved regions flanking either the base-pair polymorphism or the microsatellite repeat to construct primers for amplifying the relevant DNA portions. In some cases, two sets of PCR primers will be required: one to amplify the general region of the DNA of interest and the other to perform OLA determination of the base-pair polymorphisms. When the microsatellite regions are amplified using the primers set forth herein, representing conserved regions at either end of the repeats intervening sequences of varying lengths result. In the case of each marker, one of the alleles found in the tested population has a higher frequency in individuals known to be affected by HH than in the general population. Each individual marker cannot be completely determinative, since any particular allele associated with HH is also present in at least some normal individuals or chromosomes. However, the presence of the HH-associated allelic form of even one marker indicates an enhanced probability that the subject carries the mutation. By using multiple markers, at least two, preferably at least three, and more preferably at least four, or a greater multiplicity of such alleles to determine a characteristic genotype, this problem is reduced to the extent that substantial predictive power is obtained. The frequency of the occurrence of the characteristic genotype combination of the alleles most commonly encountered in HH-affected individuals has so far reduced to zero in normal subjects; as more individuals are tested, small numbers in the normal population will be found eventually to share some of these genotypes. This is to be expected since approximately one in fifteen individuals is a carrier of the common ancestral mutation and is clinically normal and will remain so.

To standardize the notation, the markers which are microsatellite repeat alleles are denoted by the marker name followed by a colon and the number of nucleotides in the allele found at a higher frequency in HH subjects. Thus, the notation 1A2:239 indicates that the marker bracketed by SEQ ID NO:1 and SEQ

ID NO:2 described below has 239 nucleotides which represents the sum of the nucleotides intervening between the two identified primer sequences in the HH genotype plus the nucleotides included in the relevant primers exemplified below, i.e., SEQ ID NO:1 and SEQ ID NO:2. Similarly, 24E2:245 reflects 245 nucleotides between and including the two primers identified as SEQ ID NO:5 and SEQ ID NO:6 in the HH genotype. The location of the intervening nucleotides is shown for the repeat markers as an underlined sequence in Figures 1A-1W.

Shown in Figure 1 are various-length nucleotide sequences either side of the markers described herein. Each portion of the figure shows the relevant sequence surrounding each polymorphism. These sequences are of sufficient length that it is convenient to provide them in computer-readable medium. The medium would include those known in the art such as floppy disks, hard disks, random access memory (RAM), read only memory (ROM), and CD-ROM. The invention is also directed to computer-readable media having recorded thereon the nucleotide sequence depicted with respect to each marker as set forth in Figure 1 or a portion of each such sequence wherein said portion is novel -- i.e., does not currently exist in computer-readable form.

In addition to the microsatellite repeat markers described above, three single base-pair polymorphisms have been found in which one allele is present in high proportion on chromosomes of affected individuals. These base-pair polymorphisms designated HHP-1, HHP-19 and HHP-29, were discovered in the course of sequencing the relevant portion of chromosome 6 derived from affected as compared to unaffected individuals. HHP-1 is about 80,000 base pairs centromere-proximal to the marker D6S105; HHP-19 is about 110,000 base pairs centromere-proximal to the marker D6S105, HHP-29 is about 185,000 base pairs centromere-proximal to the marker D6S105. The precise nature of the base-pair polymorphisms is set forth in the examples hereinbelow. The presence of one allele, especially in combination with any one of the characteristic allelic variants among the microsatellite repeat markers characterized herein or characterized in the prior art indicates the presence of the common HH mutation.

To perform the diagnostic test, a suitable genomic DNA-containing sample from a subject is obtained and the DNA extracted using conventional techniques. DNA can be prepared, for example, simply by boiling the sample in SDS. Most typically, a blood sample, a buccal swab, a hair follicle preparation or a nasal aspirate is used as a source of cells to provide the DNA. The extracted DNA is then subjected to amplification, for example, using the polymerase chain reaction (PCR) according to standard procedures. Sequential amplification is conducted with various pairs of primers and the amplified DNA is recovered after each amplification, or, in the alternative, the DNA sample can be divided into aliquots and each aliquot amplified separately if sufficient DNA is available. The size of the insert of the amplified marker which is a microsatellite repeat is then determined using gel electrophoresis. See Weber and May Am J Hum Genet (1989) 44:388-339; Davies, J. *et al.* Nature (1994) 371:130-136. The presence or absence of the single base-pair polymorphism is determined by conventional methods including manual and automated fluorescent DNA sequencing, primer extension methods (Nikiforov, T.T. *et al.* Nucl Acids Res (1994) 22:4167-4175); oligonucleotide ligation assay (OLA) (Nickerson, D.A. *et al.* Proc Natl Acad Sci USA (1990) 87:8923-8927); allele-specific PCR methods (Rust, S. *et al.* Nucl Acids Res (1993) 6:3623-3629); RNase mismatch cleavage, single strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and the like.

As will further be described in Example 1, one genotype associated with HH is defined by the following alleles 19D9:205; 18B4:235; 1A2:239; D6S306:238; 1E4:271; 24E2:245; additional alleles that may be included are 2B8:206 and D6S258:199. The absence of this genotype indicates the absence of the ancestral HH gene mutation in the genome of said individual and the presence of said genotype indicates the presence of said HH gene mutation.

In addition to the genotype described above, genotypes characterized by the presence of the allele associated with the HHP-1, the HHP-19 or HHP-29 single base-pair polymorphism in combination with any of the HH-associated

allelic variants among the microsatellite repeat markers also characterizes an individual whose genome contains the common HH mutation. If desired, the particular allele associated with the common HH mutation can be designated in a manner analogous to the notation used in connection with the microsatellite repeat markers hereinabove. Thus, the HH-associated alleles for the herein base-pair polymorphisms are HHP-1:A, HHP-19:G, and HHP-29:G. (See Example 4.)

The alleles associated with the single base-pair polymorphisms HHP-1, HHP-19 and HHP-29 have, to date, been observed to be in complete linkage disequilibrium. Thus, the determination that one of these alleles is present or absent specifies the presence or absence of the other. For example, an individual who is homozygous for the HHP-1:A allele is also homozygous for the HHP-19:G and the HHP-29:G alleles.

As will be evident from the above description, individual chromosomes are not necessarily isolated, the particular set of markers associated with a single chromosome can be, but need not be, determined in determining genotypes. Strictly speaking the presence of alleles associated with the common HH mutation should accompany it on the same chromosome. However, the presence of the diagnostic genotype *per se* is sufficient to indicate the likelihood that the subject carries the common HH mutation even if the chromosomes are not separated in the analysis.

It is apparent, however, that the various genotypes can distinguish between heterozygous carriers and individuals homozygous with respect to the ancestral HH mutation. That is, the presence of more than one genotype can be detected in a single individual even though total DNA is sampled.

The diagnostic methods described below have additional advantages. Although the prior art methods for identification of the presence of the genetic mutation associated with HH are invasive, current medical practice requires investigation of immediate relatives to discover any previously unsuspected cases so that preventive phlebotomy can be initiated (Bothwell, T.H. *et al.* in The

Metabolic Basis of Inherited Disease, McGraw Hill, New York, 1995, pp. 2237-2269; Edwards, C.Q. *et al.* New Engl J Med (1993) 328:1616-1620). The methods described in the present invention will be capable of detecting other cases with high accuracy in this family context, even in the event that HH is caused by a nonancestral mutation in this family. This is true because other family members who are affected will carry the same genotype as the affected member (even if these genotypes are not any of the ancestral types listed herein). Thus, these markers will still identify other family members homozygous for the HH gene.

The presence of the HH genotype also has predictive power with respect to certain therapeutic regimes where it is understood that the effectiveness of these regimes is related to the HH genotype. For example, it has been disclosed that the potential for hemochromatosis interferes with the effectiveness of interferon treatment of hepatitis C (Bacon, B. Abstracts of the Fifth Conference of the International Association for the Study of Disorders of Iron Metabolism (1995) 15-16. Thus, knowledge of the status of the genotype of the subject with respect to the HH mutation provides guidance in designing therapeutic protocols for conditions affected by disorders of iron metabolism, particularly liver conditions. As the correlations between treatment regimens and iron metabolism continue to become established, the diagnostic methods of the invention provide a useful tool in designing therapeutic protocols consistent with the presence or absence of the common HH mutation.

The following examples are intended to illustrate but not to limit the invention.

Example 1

Identification of Markers for HH

Clones containing the relevant sequences were retrieved in a genome walking strategy beginning with the previously described markers D6S306,

D6S105 and D6S258. Standard chromosome-walking techniques are described in Sambrook, J. *et al.* Molecular Cloning - A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, New York (1989) and in Dracopoli, H. *et al.* eds. Current Protocols in Human Genetics, J. Wiley & Sons, New York (1994).

The DNA sequence of the human genome in the region of the HH mutated gene was determined. A genomic 3 kb clone library was prepared by sonication of cosmid and phage P1 clones. The sonicated genomic DNA was end-repaired, BstXI adapters were added and the fragments were ligated into pOT2. Resulting clones were subjected to transposon-mediated directed DNA sequencing. See Strathman, *et al.* Proc Natl Acad Sci USA (1991) 88:1247-1250.

As a result of determining the sequence of some of the DNA in this region, the presence of 10 previously unknown microsatellite repeat elements (consisting of repeating di-, tri- and tetranucleotide repeats, most commonly the dinucleotide $(CA)_n$) was noted. The length of these repeats is typically polymorphic in the human population and thus different lengths represent different alleles which are inherited in a Mendelian fashion. This permits them to be used as genetic markers (Weber, J. *et al.* Am J Hum Genet (1989) 44:388-396).

Since the genomic sequence surrounding the repeats was thus available, PCR primers that flank the repeats and represent conserved sequences can be designed. Table 1 shows the names of these sequence repeat markers and the corresponding DNA sequences of the flanking PCR primers.

Table 1

Markers in the HH Region on Chromosome 6p2.1

Marker Name	Primer Sequences $5' \rightarrow 3'$	SEQ ID NO

Table 1

Markers in the HH Region on Chromosome 6p2.1

Marker Name	Primer Sequences 5' → 3'	SEQ ID NO
1A2	AGT CAT CTG AAG AGT TGG GCA TGT CTT CTT TGT TAA GG	1 2
1E4	AAT CAA GTT CTA GCA CC GAA TGG AGG GAG TTT ATG	3 4
24E2	CTG TTT ACA TCG GGA AGA GAC TTA G CGA ATA GTG TTA AAA TTT AAG CTA GGG CTG	5 6
18B4	CTATGGATCTTATTGTGCCT TACAGGGAGTCTACAGGACC	7 8
19D9	AGACTTTCAAAAACTCACAATCAC GATAGAACATTAGCTTAGACATGG	9 10
2B8	GAAGGACTTGAAAGGAATAC GGAATTTGAAGCTACAGTG	11 12
3321-1	TTTGGGTTTATTGCCTGCCTCC AACAATGCCCTTCCTTTC	13 14
4073-1	AACCCAGAATCACATCTAGTGAGG TGATGCATATGGCCTTTTCTTTCTC	15 16
4440-1	ATGCTGTTATTTTTTCACTTTTTCCTG AGTACTCTGTTGCAGTGAGAGATG	17 18
4440-2	ATAGACACTGACATCATCCCTACC GTTTTCTCTCCAGGACAAATTTACC	19 20
731-1	GTTGGAGAGATAGGTGTTCTTTTCC CCTGTACTACCCAAGCACCTGC	31 32
5091-1	GGGTTAAATCTCATCCCGCGGC GGCTGCAGGAACTGGGGAGGG	33 34
3216-1	ACTCCAGCCTGGGCAATAGAGC ACTCTTCGGTGTGGCAATCCGC	35 36
4072-2	AATAATGTTAAGTAACAAACTAGAGTAC ACTCCAGCCTGGGCAATAGAGC	37 38
950-1	TCATAAAACTCTACCAACATATCTCC GGAATTCCTGTGTGAAGAAATAAACC	39 40
950-2	TTCTGCCAACCAAATTCAAGACTATC GCAGAAAAATGTTTAATTCAGGAGGG	41 42
950-3	AGTCTTTGTGTAAGCATATATAAGCC CATACCGTGCAGAATCTGAACTGG	43 44
950-4	AAAACATATAAGTGTTTTCAGAGAAGG GTCTAGGCCATTTTGTCATTTAGGC	45 46
950-5	CCCCTCCTCCTGCTTTTTCTCC TTATTTACATTTGAAGGAATGGAAACC	47 48
950-6	GCTTTTCAATCACTGCTTCCCTCC AGAGAAGGAGTGGACATATGGTGG	49 50

Table 1

Markers in the HH Region on Chromosome 6p2.1

Marker Name	Primer Sequences 5' → 3'	SEQ ID NO
950-8	GGCTTCATTAATTACATTGTTTTTCAAG CAGCCTGGGAGACAGAGTGAGG	51 52
63-1	CCACAACCAGATGTCTCCTGCG GCACCTTCCAGAGAAGTTAGCCG	53 54
D6S306	TTTACTTCTGTTGCCTTAATG TGAGAGTTTCAGTGAGCC	21 22
D6S258	GCAAATCAAGAATGTAATTCCC CTTCCAATCCATAAGCATGG	23 24
D6S105	GCCCTATAAAATCCTAATTAAC GAAGGAGAATTGTAATTCCG	25 26
D6S1001	TCTGGGATTCCTGTCCAATG CCTGACATATAGTAGGCACTC	27 28
D6S464	TGCTCCATTGCACTCC CTGATCACCCTCGATATTTTAC	29 30
65-1	TGT ATG GGG TAA ATC CAA GTT GCC ACA AAT AGA GAA AGT TAT CTT TAG AGG	55 56
65-2	TGT GTT TCA GTC AGC TAT TGC TCC TGT ACT TAC ATC TTA AGG TAC AGC C	57 58
63-2	CTC CAG GCT GGC CGA CAA AAG C ATG TAT ATT ACA GCT TTT ATA ATT GTC C	59 60
63-3	TCA CAA TCA TTT TTT GAT AGC CTA TCC AGC CTT TAG GTA TTT TCA CAC TTG C	61 62
373-8	CCA GCT CAT TAG TCT TTC TTG TAG C ACT GAG ATC ATT TAC TGT TAC TAG AC	63 64
373-29	GTT CAT TCC ATT TCA GGC ATA TTC G ATT AGT AGA AAG ATT TAG AGT AAA TGC	65 66
68-1	CTT GAT TCT GAT TCA CAT TTG ACT CC TAT TAT ATG TCA TCA GAA GTA TTA GGG	67 68
241-6	GCA ATG ACA CCC TCC CAT CAC C TAT CAG ATG ACA TTT TAG GAG ACC C	69 70
241-29	CCT ATA CAA TAA ATC TAT AAA AAG TGG G ATT CCT GTG TCT TTC CAG AAC ACC	71 72

As shown in Table 1, a large number of new markers were identified; with respect to the prior art markers D6S306 D6S258, D6S105, D6S1001, and D6S464, the appropriate primer oligonucleotides are also determined. As will be

shown in Example 2, the alleles associated with HH for both the new markers and four known markers have also been determined.

Example 2

Association of Alleles with the Presence of HH

Total genomic DNA from families represented in the CEPH collection (Dausset, J. *et al.* Genomics (1990) 6:575-577) was used as a substrate for amplification with the 14 pairs of primers representing the markers in Table 1. None of the individuals in the CEPH collection is known to have HH; thus, the results in these individuals indicate the frequencies of the various alleles in the normal population. These results are shown as the "% Normals" in Table 2.

Table 2

Allele Distribution for HH Markers

Marker Name	Allele Size (base pr.)	% Normals	% HH
1A2	237	2	0
	239	46	77
	241	35	21
	243	16	3
1E4	257	1	0
	261	1	0
	265	4	0
	267	10	7
	269	31	13
	271	28	70
	273	9	5
	275	9	0
	277	3	0
	279	1	0
	281	1	0
	283	3	5
	285	1	0
	287	1	0

Table 2

Allele Distribution for HH Markers

Marker Name	Allele Size (base pr.)	% Normals	% HH
24E2	251	2	0
	235	6	5
	237	1	0
	239	1	0
	241	3	0
	243	18	9
	245	63	82
	247	9	4
18B4	231	1	0
	233	23	12
	235	42	78
	237	25	10
	239	8	0
19D9	183	1	0
	185	1	0
	199	9	1
	201	2	0
	203	15	12
	205	63	87
2B8	198	0	0
	202	0	4
	204	4	1
	206	14	67
	210	27	10
	214	11	6
	216	2	0
	218	3	1
	220	5	8
	226	2	0
	228	10	0
	230	4	0
	232	3	3
	234	3	0
3321-1	195	21	12
	197	71	81
	199	8	8
	201	1	0

Table 2

Allele Distribution for HH Markers

Marker Name	Allele Size (base pr.)	% Normals	% HH
4073-1	180	3	2
	182	49	82
	184	12	5
	186	21	5
	188	7	4
	190	3	1
	192	1	0
	212	1	0
	238	1	0
4440-1	176	10	13
	178	47	25
	180	38	61
	182	3	1
4440-2	139	58	82
	141	2	0
	143	9	4
	145	0	1
	149	7	1
	151	1	0
	155	5	3
	157	4	4
	159	8	4
	161	2	1
	163	3	0
	165	1	0
	167	1	0
63-1	159	0.7	0
	157	4.3	1
	155	3.6	1
	153	0.0	3
	151	13.6	76
	149	0.0	1
	147	0.0	2
	145	0.7	1
	143	1.4	0
	141	21.0	3
	139	33.0	9
	137	0.7	0
	135	20.0	5
	133	0.7	0

Table 2

Allele Distribution for HH Markers

Marker Name	Allele Size (base pr.)	% Normals	% HH
D6S464	202	4	1
	204	6	3
	206	52	84
	208	2	0
	210	8	3
	214	2	0
	216	13	7
	218	2	0
	220	2	1
	222	2	0
	224	8	1
D6S306	230	4	0
	234	2	3
	238	54	74
	240	22	12
	244	11	10
	246	6	0
	248	2	0
D6S258	189	11	5
	193	2	0
	197	30	12
	199	33	72
	201	6	7
	203	2	2
	205	6	1
	207	6	0
D6S105	116	2	0
	122	2	1
	124	13	64
	126	8	3
	128	39	17
	130	14	5
	132	11	8
	134	5	3
	136	3	0
	138	3	0

Table 2

Allele Distribution for HH Markers

Marker Name	Allele Size (base pr.)	% Normals	% HH
D6S1001	176	18	8
	178	12	4
	180	40	79
	182	11	4
	184	4	0
	186	1	0
	188	2	0
	190	5	4
	192	6	1
	196	1	0
	200	2	0
65-1	218	1	0
	216	6	1
	214	8	1
	212	11	3
	210	33	8
	208	31	11
	206	8	72
	204	1	3
	202	1	2
	198	0	1
65-2	173	1	0
	169	9	3
	167	3	3
	165	0	1
	163	1	1
	161	45	12
	159	38	81
	151	1	1
	141	1	0
	131	1	0
63-2	133	24	5
	131	24	7
	129	2	1
	127	4	0
	123	6	2
	119	0	1
	117	0	1
	113	41	85

Table 2

Allele Distribution for HH Markers

Marker Name	Allele Size (base pr.)	% Normals	% HH
63-3	171	3	1
	169	49	90
	167	49	7
373-8	163	0	1
	161	2	1
	159	1	1
	157	5	1
	155	12	5
	153	29	12
	151	17	69
	149	21	7
	147	11	5
	145	1	0
	139	0	1
373-29	117	0	1
	115	1	4
	113	5	55
	111	1	7
	109	17	6
	107	20	6
	105	7	1
	103	48	19
	101	1	0
	83	0	1
68-1	171	1	0
	169	10	12
	167	52	59
	165	1	0
	163	35	29
241-6	115	1	0
	113	1	1
	109	4	0
	107	27	5
	105	24	80
	103	10	2
	101	6	3
	99	8	1
	95	0	1
	93	18	7
	87	0	1

Table 2

Allele Distribution for HH Markers

Marker Name	Allele Size (base pr.)	% Normals	% HH
241-29	121	0	1
	119	0	1
	117	20	5
	115	27	4
	113	11	82
	111	0	1
	103	42	7

With respect to HH, the haplotypes for many of the single chromosomes were obtained from the DNA of cell hybrid lines, each of which contained a single chromosome 6 from an HH-affected individual (Shay, J.W. Techniques in Somatic Cell Genetics, Plenem, New York, 1982). These results are shown as "% HH" in Table 2. For each marker, generally one allele was more common in HH chromosomes as compared to normal individuals.

Example 3

Determination of Haplotypes Associated with HH

Table 3 shows a compilation of haplotypes assembled from the alleles most commonly occurring in HH chromosomes. Haplotype A assembles six of the ten markers; haplotypes B and C expand the assembly with one additional marker each and haplotype D adds two additional markers for a total of eight.

Table 3

6p Marker Haplotype Associations with HH

Markers	D6S258	19D9	18B4	1A2	2B8	D6S306	1E4	24E2
Haplotype A		205	235	239		238	271	245
Haplotype B		205	235	239	206	238	271	245
Haplotype C	199	205	235	239		238	271	245

Haplotype D	199	205	235	239	206	238	271	245

Table 4 shows the distribution of these haplotypes as determined in 74 hemochromatosis chromosomes and 56 chromosomes from unaffected individuals. Inheritance patterns could be used to associate the haplotypes with particular chromosomes in the CEPH individuals and HH individuals.

Table 4
Frequency of Haplotypes in Affected and Unaffected: (%)

	Individuals		Chromosomes	
	Affected	Unaffected	Affected	Unaffected
A	89	0	68	0
B	86	0	58	0
C	84	0	61	0
D	81	0	51	0

Table 4 clearly shows that none of the haplotypes A-D occurs in unaffected individuals or in unaffected chromosomes tested to date. A very high percentage of individuals affected by HH contains haplotype A and significant numbers contain B-D. Indeed, these haplotypes are present on a majority of chromosomes from HH-affected individuals.

Example 4

Single Base-Pair Polymorphisms

In the course of sequencing the HH region of genomic DNA prepared as described in Example 1, and by comparing the sequences obtained for DNA from affected as compared to unaffected individuals, three single base-pair polymorphisms were found and designated HHP-1, HHP-19 and HHP-29 as follows:

HHP-1

Unaffected sequence:

TCTTTTCAGAGCCACTCACGCTTCCAGAGAAAGAGCCT

Affected sequence:

TCTTTTCAGAGCCACTCACACTTCCAGAGAAAGAGCCT

HHP-19

Unaffected sequence:

ATATATCTATAATCTATATTTCTTAAGACAATTAAGAATG

Affected sequence:

ATATATCTATAATCTATATTTCTTGAGACAATTAAGAATG

HHP-29

Unaffected sequence:

TTGGGGATTTTATAGATTTTATTTTTAAAAAATGTTTAATCTTT GT

Affected sequence:

TTGGGGATTTTATAGATTTTAGTTTTAAAAAATGTTTAATCTTT
GT

The presence or absence of these single base-pair sequence differences can, of course, be determined in the same DNA samples as those which provide information on the $(CA)_n$ repeat alleles by use of the appropriate primers for amplification and sequencing. Figure 2 shows the sequences of primers used for amplification and sequencing of the above three base-pair polymorphisms. The amplification primers for HHP-1 are labeled AG77 and AG78; the amplification primers for HHP-19 are labeled AG110 and AG111; and the amplification primers for HHP-29 are labeled AG165 and AG166. The primers used in the sequence determination by OLA are designated, for HHP-1, AG64, AG62 and AG63; for HHP-19, AG143, AG144 and AG145; and for HHP-29 are designated AG190, AG191 and AG192. As indicated in the sequences shown, "bio" indicates biotin coupling; "dig" indicates coupled digoxygenin.

Table 5 shows the frequency of these point mutations in affected and unaffected chromosomes:

Table 5

Frequencies of Alleles as % of Chromosomes Tested

		Affected Chromosomes	Random Chromosomes
HHP-1	A	64%	6%
	G	36%	94%
HHP-19	G	64%	6%
	A	36%	94%
HHP-29	G	64%	6%
	T	36%	94%

The allele in HHP-1:A occurs in 64% of the affected chromosomes; its occurrence at 6% in random chromosomes approximates the estimated frequency of the common HH mutation in the population. As noted hereinabove, according to the results obtained to date, the presence of HHP-1:A is associated with the presence of HHP-19:G and HHP-29:G.

Claims

1. A method to determine the presence or absence of the common hereditary hemochromatosis (HH) gene mutation in an individual, which method comprises:

obtaining genomic DNA from said individual; and

assessing said DNA for the presence or absence of the HH-associated allele of the base-pair polymorphism designated herein HHP-1, HHP-19 or HHP-29;

wherein the absence of said allele indicates the likely absence of the HH gene mutation in the genome of said individual and the presence of said allele indicates the likely presence of said HH gene mutation in the genome of the individual.

2. The method of claim 1 wherein said assessing step further includes determining a genotype by additionally assessing said DNA for the presence or absence of any one of the following alleles defined by markers having microsatellite repeats, wherein the number subsequent to the colon indicates the number of nucleotides between and including the flanking primers when the primers are those exemplified herein:

19D9:205;
18B4:235;
1A2:239;
1E4:271;
24E2:245;
2B8:206;
3321-1:197;
4073-1:182;
4440-1:180;

4440-2:139;
731-1:177;
5091-1:148;
3216-1:221;
4072-2:148;
950-1:142;
950-2:164;
950-3:165;
950-4:128;
950-5:180;
950-6:151;
950-8:137;
63-1:151;
63-2:113;
63-3:169;
65-1:206;
65-2:81;
373-8:151;
373-29:109;
68-1:167:
241-6:105;
241-29:113;
D6S464:206;
D6S258:199;
D6S265:122;
D6S105:124;
D6S306:238; and
D6S1001:180.

wherein the presence of the genotype corresponding to said HHP-1, HHP-19 or HHP-29 HH-associated allele in combination with said at least one microsatellite repeat allele indicates the presence of said HH gene mutation in the

genome of said individual, and the absence of said genotype indicates the absence of said HH gene mutation in the genome of said individual.

3. The method of claim 1 wherein said assessing of the DNA is performed by a process which comprises subjecting said DNA to amplification using primers flanking at least one of said base-pair polymorphisms.

4. The method of claim 2 wherein said assessing of the DNA is performed by a process which comprises subjecting said DNA to amplification using primers flanking at least one of said base-pair polymorphisms and subjecting said DNA to amplification using primers flanking at least one of said microsatellite repeat allele markers.

5. A set of primers for the conduct of oligonucleotide ligation assay determination of the presence or absence of an HH-associated allele of a base-pair polymorphism,

wherein the base-pair polymorphism is HHP-1 and the primers are oligonucleotides comprising the nucleotide sequences SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35; and/or

wherein the base-pair polymorphism is HHP-19 and the primers are oligonucleotides comprising the nucleotide sequences SEQ ID NO:38, SEQ ID NO:39 and SEQ ID NO:40; and/or

wherein the base-pair polymorphism is HHP-29 and the primers are oligonucleotides comprising the nucleotide sequences SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45.

6. A kit for the detection of the presence or absence of the HH-associated allele of the base-pair polymorphism designated herein HHP-1, HHP-19 or HHP-29 which kit comprises at least one of the primer sets of claim 5 and optionally further includes primers for amplifying the DNA containing the base-pair polymorphism.

7. A method to determine the likelihood of the presence or absence of the common hereditary hemochromatosis (HH) gene mutation in an individual, which method comprises:

obtaining genomic DNA from said individual; and

assessing said DNA for the presence or absence of the genotype defined by the presence of at least one nonoptional marker comprising the following microsatellite repeat alleles, wherein the number subsequent to the colon indicates the number of nucleotides between and including the flanking primers when the primers are those exemplified herein:

19D9:205;
18B4:235;
1A2:239;
1E4:271;
24E2:245;
2B8:206;
3321-1:197;
4073-1:182;
4440-1:180;
4440-2:139;
731-1:177;
5091-1:148;
3216-1:221;
4072-2:148;
950-1:142;

950-2:164;
950-3:165;
950-4:128;
950-5:180;
950-6:151;
950-8:137;
63-1:151;
63-2:113;
63-3:169;
65-1:206;
65-2:81;
373-8:151;
373-29:109;
68-1:167:
241-6:105; and
241-29:113;

optionally in the presence of at least one optional marker comprising the following microsatellite repeat alleles:

D6S464:206;
D6S306:238;
D6S258:199;
D6S265:122;
D6S105:124; and
D6S1001:180,

wherein the absence of said genotype indicates a likelihood of the absence of the HH gene mutation in the genome of said individual and the presence of said genotype indicates a likelihood of the presence of said HH gene mutation in the genome of said individual.

8. The method of claim 7 wherein said genotype includes at least two of said nonoptional markers.

9. The method of claim 8 wherein said genotype includes at least three of said nonoptional markers.

10. The method of claim 9 wherein said genotype includes at least four of said nonoptional markers.

11. The method of claim 7 wherein said genomic DNA is prepared from a sample of blood or buccal swab from said individual.

12. The method of claim 7 wherein said assessing of the DNA is performed by a process which comprises subjecting said DNA to amplification using primers flanking at least one of said $(CA)_n$ repeat allele markers.

13. A DNA primer pair that flanks a marker selected from the group consisting of 19D9; 18B4; 1A2; 1E4; 24E2; 2B8; 3321-1; 4073-1; 4440-1; 4440-2; 731-1; 5091-1; 3216-1; 4072-2; 950-1; 950-2; 950-3; 950-4; 950-5; 950-6; 950-8; 63-1; 63-2; 63-3; 65-1; 65-2; 373-8. 373-29; 68-1; 241-6; and 241-29.

14. The DNA primer pair of claim 13 which flanks the marker 19D9 and wherein the primers in said pair have the nucleotide sequences SEQ ID NO:9 and SEQ ID NO:10; and/or

wherein the marker is 18B4 and wherein the primers in said pair have the nucleotide sequences SEQ ID NO:7 and SEQ ID NO:8; and/or

wherein the marker is 1A2 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:1 and SEQ ID NO:2; and/or

wherein the marker is 1E4 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:3 and SEQ ID NO:4; and/or

wherein the marker is 24E2 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:5 and SEQ ID NO:6; and/or

wherein the marker is 2B8 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:11 and SEQ ID NO:12; and/or

wherein the marker is 3321-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:13 and SEQ ID NO:14; and/or

wherein the marker is 4073-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:15 and SEQ ID NO:16; and/or

wherein the marker is 4401-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:17 and SEQ ID NO:18; and/or

wherein the marker is 4440-2 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:19 and SEQ ID NO:20; and/or

wherein the marker is 731-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:31 and SEQ ID NO:32; and/or

wherein the marker is 5091-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:33 and SEQ ID NO:34; and/or

wherein the marker is 3216-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:35 and SEQ ID NO:36; and/or

wherein the marker is 4072-2 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:37 and SEQ ID NO:38; and/or

wherein the marker is 950-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:39 and SEQ ID NO:40; and/or

wherein the marker is 950-2 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:41 and SEQ ID NO:42; and/or

wherein the marker is 950-3 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:43 and SEQ ID NO:44; and/or

wherein the marker is 950-4 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:45 and SEQ ID NO:46; and/or

wherein the marker is 950-5 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:47 and SEQ ID NO:48; and/or

wherein the marker is 950-6 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:49 and SEQ ID NO:50; and/or

wherein the marker is 950-8 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:51 and SEQ ID NO:52;

wherein the marker is 63-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:53 and SEQ ID NO:54;

wherein the marker is 65-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:55 and SEQ ID NO:56;

wherein the marker is 65-2 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:57 and SEQ ID NO:58;

wherein the marker is 63-2 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:59 and SEQ ID NO:60;

wherein the marker is 63-3 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:61 and SEQ ID NO:62;

wherein the marker is 373-8 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:63 and SEQ ID NO:64;

wherein the marker is 373-29 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:65 and SEQ ID NO:66;

wherein the marker is 68-1 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:67 and SEQ ID NO:68;

wherein the marker is 241-6 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:69 and SEQ ID NO:70; and/or

wherein the marker is 241-29 and wherein the primers in said pairs have the nucleotide sequences SEQ ID NO:71 and SEQ ID NO:72;

15. A DNA primer pair for the marker D6S258 wherein the primers in said pair have the nucleotide sequences SEQ ID NO:21 and SEQ ID NO:22; and/or

for the marker D6S306 wherein the primers in said pair have the nucleotide sequences SEQ ID NO:23 and SEQ ID NO:24; and/or

for the marker D6S105 wherein the primers in said pair have the nucleotide sequences SEQ ID NO:25 and SEQ ID NO:26; and/or

for the marker D6S1001 wherein the primers in said pair have the nucleotide sequences SEQ ID NO:27 and SEQ ID NO: 28; and/or

for the marker D6S464 wherein the primers in said pair have the nucleotide sequences SEQ ID NO:29 and SEQ ID NO:30.

16. A kit for the determination of the presence or absence of an HH gene mutation in an individual which kit includes at least one DNA primer pair that flanks the marker selected from the group consisting of

19D9;
18B4;
1A2;
1E4;
24E2;
2B8;
3321-1;
4073-1;
4440-1;

4440-2;
731-1;
5091-1;
3216-1;
4072-2;
950-1;
950-2;
950-3;
950-4;
950-5;
950-6;
950-8;
63-1:115;
63-2:113;
63-3:169;
65-1:206;
65-2:81;
373-8:151;
373-29:109;
68-1:167:
241-6:105; and
241-29:113.

17. The kit of claim 16 which further includes DNA primers flanking the marker D6S306, and/or which further comprises DNA primers for the marker D6S258, and/or which further comprises DNA primers for the marker D6S105, and/or which further comprises DNA primers for the marker D6S265, and/or which further comprises DNA primers for the marker D6S1001, and/or which further comprises DNA primers for the marker D6S464.

18. The kit of claim 16 wherein said primer pairs have the nucleotide sequences set forth as follows:

19D9: SEQ ID NO:9 and SEQ ID NO:10,

18B4: SEQ ID NO:7 and SEQ ID NO:8,

1A2: SEQ ID NO:1 and SEQ ID NO:2,

1E4: SEQ ID NO:3 and SEQ ID NO:4,

24E2: SEQ ID NO:5 and SEQ ID NO:6,

2B8: SEQ ID NO:11 and SEQ ID NO:12,

3321-1: SEQ ID NO:13 and SEQ ID NO:14,

4073-1: SEQ ID NO:15 and SEQ ID NO:16,

4440-1: SEQ ID NO:17 and SEQ ID NO:18,

4440-2: SEQ ID NO:19 and SEQ ID NO:20,

731-1: SEQ ID NO:31 and SEQ ID NO:32,

5091-1: SEQ ID NO:33 and SEQ ID NO:34,

3216-1: SEQ ID NO:35 and SEQ ID NO:36,

4072-2: SEQ ID NO:37 and SEQ ID NO:38,

950-1: SEQ ID NO:39 and SEQ ID NO:40,

950-2: SEQ ID NO:41 and SEQ ID NO:42,

950-3: SEQ ID NO:43 and SEQ ID NO:44,

950-4: SEQ ID NO:45 and SEQ ID NO:46,

950-5: SEQ ID NO:47 and SEQ ID NO:48,

950-6: SEQ ID NO:49 and SEQ ID NO:50,

950-8: SEQ ID NO:51 and SEQ ID NO:52;

63-1: SEQ ID NO:53 and SEQ ID NO:54;

65-1: SEQ ID NO:55 and SEQ ID NO:56;

65-2: SEQ ID NO:57 and SEQ ID NO:58;

63-2: SEQ ID NO:59 and SEQ ID NO:60;

63-3: SEQ ID NO:61 and SEQ ID NO:62;

373-8: SEQ ID NO:63 and SEQ ID NO:64;

373-29: SEQ ID NO:65 and SEQ ID NO:66;

68-1: SEQ ID NO:67 and SEQ ID NO:68;

241-6: SEQ ID NO:69 and SEQ ID NO:70; and

241-29: SEQ ID NO:71 and SEQ ID NO:72.

19. The kit of claim 17 wherein the DNA primers for marker D6S258 have SEQ ID NO:21 and SEQ ID NO:22; and the DNA primers for marker D6S306 have SEQ ID NO:23 and SEQ ID NO:24; and the DNA primers for marker D6S105 have SEQ ID NO:25 and SEQ ID NO:26; and the DNA primers for marker D6S1001 have SEQ ID NO:27 and SEQ ID NO:28.

20. A method to evaluate the responsiveness of a subject to interferon treatment for hepatitis C, which method comprises determining the presence or absence of the common hereditary hemochromatosis gene in said subject according to the method of claim 1.

21. A method to evaluate the responsiveness of a subject to interferon treatment for hepatitis C, which method comprises determining the presence or absence of the common hereditary hemochromatosis gene in said subject according to the method of claim 7.

22. A method to determine the presence or absence of a genome homozygous for a hereditary hemochromatosis gene mutation in a subject related by blood to an individual diagnosed as afflicted with hereditary hemochromatosis which method comprises comparing the genotype of said subject as determined by the method of claim 2 with the similarly obtained genotype of said individual.

23. A method to determine the presence or absence of a genome homozygous for a hereditary hemochromatosis gene mutation in a subject related by blood to an individual diagnosed as afflicted with hereditary hemochromatosis which method comprises comparing the genotype of said subject as determined by the method of claim 7 with the similarly obtained genotype of said individual.

24. A computer-readable medium having recorded thereon the nucleotide sequence depicted in any of Figures 1A-1W or a novel fragment thereof.

25. A primer useful for amplification of DNA designed on the basis of the DNA sequence set forth in any of Figure 1A-1W.

1260 bp around the polymorphism 1001

Fig 1A

[illegible]
[illegible]
[illegible]
AGAC ATTTGCAAAAATGCATCAGTAATAACTTAATTCATCACACTGGATAAAATTTC
ATCTGGTTAAGATTTCATCACTTAAGTAAAGAGGAAGAAGAGGTTTTTATATTGATA
TTGAAAAGTCCTTGATTGTATTGATGCCATTATTCTTATCTCTAAACATGAACTGATG
TCACCATTTCTTTATATCAGTCTCAGTTTTGATAACAAATTGACTCTCTTAAACTTCTTA
AGCAGATTGATAATTCATGCACTTTCTTTGTATCCAGTGACTCTAATCTTAAACAAATGGA
ACATAAAATACTGAACCAATTAGCAAAATGAACTGTTTCTTAAACGTTTATAACAATCTA
[illegible]
[illegible]
ACAAACACACACACAGTGGCAATTAAATATTCTGTGCCTTGAAAAGTGAGAAAGGATACAG
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CTTCATTAAACATCTGTGCAATCAGCCAGACACAAACTTGCAGACCCCCGCCTCACCACTC
TCCTGCCTCTTATCTGATAAATCTCCCAGTGCCGCAAATTCTCCCTCTAGCCCGGCTTGT
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TCCTAGATTACTGCAGCCATCTCCTGTTTGTCTCTCATTTTCCAGTATCACTCTCTTCTA
ATTTGCTGCAGCTGTAGTTAGGTTCTAAATTCCAAATTCATTCATGTATCTACTTTAAAT
AACTCAGTACTTCTTTTTTGTTTGTTTGTTTTTCATAATGACAAAACTCCTTAACATGAG
CTACAAGATCATGCATATTCTGGTCCCTATTCCTTAACTAGTCAGAGTGAATGTCATTCC

1260 bp around polymorphism 1909

Fig 1B

GTAATAAAATTATTTAGGGTTTTGGATTGTGTCAAAGCCCTATTCAGCAATGTCTACTTG
AAAATTTCATTGAAAAAGTAACTTAAATAAAGTAGCTATTTGAAGGGCTCAGTAGGATAG
AACCCTTGTCCTTAGCATGAATCATAGTGAGAAGACACTTTTGAATATGTTTGTTTTTCT
TCTATTACCAGGAAAACATAGGATCATAAATCACAATTATTCCATATGTTTTAGAAATTA
ATCATGTGTATCTTTGCACAAGCACCATAATGCTTGTGTGTATAAATGAGTATGCATGCA
TACTTGTAAACACACAGCTTTCATACTCGCTTTTATTATTGTCACTTTTAACAGCCCCTT
ACATGAAATTTATATTTAAAAAGTGAGAACATTTATATTCATTCTGATGTATTCAGACAC
TTGTATTAAATTCTTAGCTCTACTATTTGTGGTCTGTTTGATAATGTTTCCTAATCTATC
AAATGAAAGGATTCTGAATTGATCATTTGTTTTTCAAATGTATATTCATGTTAGAATCTCA
CAAGGAGCTTTTTTCAACAAAATATTTCCAGACTTTCAAAAACTCACAATCACTGTGGTTG
GAACTTGAAACAAACATATGTGTGTCTGTGTGTGTATATATATATATATATACACACACACA
GACACACATATATATATATCTTTATGTAATTTTTAATGCCAGCTGATCAGTGAAACAGTGTTAA
GCTCAAAAATTTTAATGATGTCATTTTTCCATGTCTTCACTAACCTTCTCTCTTCTCCTTT
TCTCTCTTTTCCTTCCTACCAAATTTTTTTCCTACCTATTTTTTACTCTCCATTTTCTCACT
CCCCTTTAACTCATTTCCATTACACAAACTACTATTACACAAACTACTCATATAATTTTT
CCTCATCTTATCTTCCCAAAGCATAACTTCTGTCAGTCAATCCACAGTACTAAAGCATTG
ATTTATGGTTCTGTTGGATTTTAATTAGCTGTGGTCAATTTGAAAGGAGGAGAAAAAAT
GATTTGACATGTCAGATACAACATGTTATACAGATTAAATTTCAGCTGTAATCTAACTAG
TCATCAGCATTTTTATTCAGGGCTTTACAATAAGTATTCCCAAGTTCTGCCTCTGTAGGTT
TGTATTGGGTAGGTAGGAATATTTAAATGAATTTTGAAGTTTCACTTCAAGAATTATTTA
TTTCTATTAAATAAGTAAAGAAGCAGTCTCAAGAGCAGTCACTGTCACTGTGTTTTCTAG

Approximately 1 kb of sequence around the polymorphism 1A2

Fig 1C

[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]

[illegible]
[illegible]
[illegible]
AAAACAGTAAAACAATTAAAAAAT [illegible]
TAA [illegible]
TGTCATTTTTTATTA [illegible]
ACATTCTTATTAGAATGGACTAAAATCTAAAAAATGACAATACCAATTG
CTAGCAAGGATGGGAGTGGCAGAAAGTCTCATTTATTTTTTGTGAGATG
CAGAAGAGTACATCAATTTCCTGATCACTGCAATTCATTCCATACCCAC
ATAGATATTTTTTCCCCATATGTTAGGAAGCAGATCTCTATAGCCTT
CATGGACTTCTCTTTTGAGTGGAATTCACAAGGGTATCTTTAGCTTAT
[illegible]

Approximately 1180bp around polymorphism 1E4

Fig 1D

CTGTAAAGTTACCATTTTTTCTTTTTTAAATTAATAATTATCTTGAGAAAAAATATTTTGA
GATTATGAAAATATTCTGTTTTCTCATCATATTTTTTGATACTTATATTGATGTTCATCAGT
GATTCTTGCCTGCAAGAATTATTTTCTGTAGCATGTATTTTTGTATTTGTATTGCTAATTGT
ACATTTATTAATTGGAATTCTACTGTAAAGAAGAGGTGTTATTTTTCCCCCATTTGTTAT
TTGTTCAGTCATTTATTTAAACTCATATAGAGTTATAGGTATTTGTTTTATTGTATTGTT
TGTAGTCCCAATACTATCATTATTTAATTTAGTGGTAAAATTGGCCTAGATTTGAGCTTT
GGGAGCTCCTTTCAAGTTGACTCATGTATCTTTTTAACATGGCCCATCACTATTTGAGAAC
TTCTATACTCTGTGTCAGCACCAGTGTTTCTAGGGTCATCTTGAGTTTTTAGTTGCCCAG
CCCTGGAATTAGTAATTTTTGTAAGGATCCTTTGGTTCCTTTTTAGTGAGAAATATATTTGGA
AATCAAGTTCTAGGCACCAGGTGTGTTCATTGGTAGTGGATTTGTTATTGATTCCAGACTC
TCTGAGTGAACAGAGCTTACAAATAGAGTGTGTGTGTGTGTGTATATATATATATATATATATA
TATATATATATATACTGACATATACATACACATACATTTTTTATTTTATATACCTAGCTGTGTG
TGTGTATGTGTGTGTGTGTGTGACCACAGTTCATACTAATGCCTCTGATTCCAATCCAAATA
CCACATAGTATTTGCATAAACTCCCTGCATTCCTTATTTGTAGCTTCTTTGTTGAACACT
GGGAAATTTGGCTCTCATTATCCATAATATATTTAGTTATTTTCTCAATTCTAATAGACA
AATAGCTTTAGAATTGCTAATCCACACTGTTGGGAATAAGCATTTTACTAACTAGAGTAC
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GTCAAGATACTCTTTTTCCCAAAGTTAATTAGGTTAGTTTTTTTTCCTTCCTTAGCCTCT
TTAACTTGGTTTTGTTGCTCATTTGTAATACAGGTGGGTTAATTTATTATTCTCTGTATT
TCTTTTGGGTACCTCCCATTCCGGTTGACTTTAGTTATTTATTTAAATTGGAATATGTGA
AGCATTACTATGGCTATAAAAGTTAGAACACACAAAATGTTATATGTACTTAGAAAAGTG
TCACTCCCCCTCAGCCTTTCCATTCCACTAATTCTCCCATTTTTTTTATACTCTATTCCAA
ATCACCACCTCCTCCAACCCTGTGGGTAACTAATCTCATTAGTTTCTGGTTTATCATTCC

1260 bp around the polymorphism 2E2

Fig 1E

CCCAGAATTGGCCTTCCAATGCACCAAAAACTGTAATCACAAGATTTTCAAGGGTTGTGA
CACTTTTACATCAATGTTTGTACAATTCAGTGTAAACTAGACCTTTCTGATCCAGAAATCA
TCTCTTCAGTAATACACACACGCACACACACACATACATACATACACACACACACATAGAAA
CCAAGATGTAAAGGGAGCTTTTGAGAGGTTGCTTGCAAGGGTGTTAATAAAAAAAAAAAG
AATTCTCAAATTATAGGGCTTTTTAAAGACTTCAATTTTTACATAGCTTATAATTTAATTCT
CTCCAAATTGCTTTATTATTACTATTCTTAGAAAAACTATTATAGTGATCTTCAAATAAA
ATGTCGGACAGAGAACTATATCTGTTTTTCTACTGCCTAAATATATTCATTGCACAAGTCTT
AAGAACTGATCTTTTTATGAACTCTCAAAATAGCATATCCTTGAAATCTTTAAGGTCTCAA
ACATCTTAGCACTAGTCTGTATACATCTAGGAAGAGACTTAGACTTCTCTGAAACCAGAAT
AAAAGCCAGAAACAAAACATTTTGATAGATATACACATGTCCTCATCCTTACACACACAC
ACACACACACACACACACACACAAACTCCATGGCAGAAATTATTTTTCAGACAATTGTAG
ATCTAACAGAAGTATCCAAAACCTTGTCTTAATTTTCCTCTATAAGTTTAACAGCCCTAG
TTAAATTTTAACACTATTGCACATCAACACAATACTAAAATCCACAACAATTCTGACACT
CCCCAGTTTTACTTAGATCTTCTGTTGTTTCTGTACTTCCCACTTCTAAGTTGAAGTGTC
CTATTCCATCTATCAAATAAAGTTGTAGGTACATTTTAGACTGAAATCGAATGGCTGACTT
TTGACCTTTTAAAATGATTCCTCTACTGTATATATATTATCTCTCTCCTTTTAACCTCGAAA
GCACTTATAGGGGCCTAGGCGGATGGATTATGGCCTGTAATCCTAGCACTTTGGGAGGCG
[illegible]

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[illegible]

Approximately 1 kb of sequence around the polymorphism 288

Fig 1F

AGATTTCTTTTGCATTAACAAGTAATAACAAGGATTGAGTAGTAACAAGAAATTCTTTC
[illegible] TTTATCTCCTTTCTTCTT [illegible]
[illegible] TCAATTATA [illegible]
GCAGAATCCACGGTTGATTATGGTGTTTGATATACTAAAGCTGGATAATTAAACAATTTA
TTTTGGCTCTCATTCAAGCATTTGGCACTATAAAAGCATATTTGAACTTTCTAGAAAAAA
ATAAGTGCTTCTTCAGCAAGACTTCGAAGATCTTTCGTTTCATATATTGCTGAGGACCTA
CTAAGTCCTTCTAAGATCTTTCTTTTCATACATCGTTGAGGACCTATTAAATAACTGTGA
TAGAAACTGGTATGAGAACAAAAATGCCTAGTGTCTACATTCACGAACAATATTTTGGAG
GCTTTCTGGTCATGAATGCTTGATTTAGAAGGACTTGAAAGGAATACAAGTGATTGTCAAC
TCAGGAGGAATATTACATTTTTTACACTCTTGCTTTCTTTCTTTCTTTCCTCTTTCTTTC
TTTCTTTCTTTCTTTCTTTCTTTCCTCTTTCTTTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTGACAGGGTCTTGCTCTGTCACCCAGACTGAGTGCAGTGGCACAAACACGGC
TCACTGTAGCTTCAAATTCCCAGGCTCAAGCAATCCTCCCACCTCAGCCTTCTGAGTAGC
TGGGACTGTAGGCATGCACCACCATGCCTGGCTAACTTTTTAAATTTTTCGTACAGATGG
GAGTCTCACTATGTTGCTCAGGATAGTCTCAAACTCCTGGACTCAAGCAATACTCCCACC
TCTCAAAGTGCTGGGATTACAGGAAGGAGCCACTGCTCCTAGCCCCTATTTTCTTGACCT
AGGTAAACCATTGAATTCCCCCATCTCATTAAATGCCTCTTCAGCCTGCAATGCCAAAAC
ATCCCTATATTTGCTAGGTCTAACAACATATATAGAAGATGGGTCAAAATACAATCCCAA
AGTTTAATCACCCCTTACTATATTTCTGCACTCCCCTTCCCTAGCACCTTCTTCATGGCC
TCTTTAACATGTTTGTTTCTTAGTGTATAGATCAGGGGTTAACACTGGAGTGACAATT
GTGTAGAAAAGGGTAAGAAACTTGCCCTGGTCCTGGGAATAAGTATTTGCTGGCTGGAGG
TACATGTATATGATTGTACCATAGAAGAGAGAGACAACAATTAGATGCGAGCTGCAAGTG

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aagatggatgctttgttagacattcctgcag

Fig 1G

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5091-1

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agccc

Fig 1H

4440-1

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Fig 1-I

4440-2

TTATACCAGAACTGCATCTATTACAGCATAAACCAATTATATCTTTGACC
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TTGACACTGACATCATCCCTACCTTAGAAGACAAATATATGTATGTGTNT
<u>ACACACACACACACACACACACACACA</u>GAGNAGAGNCACAGATATAGTCA
ATCTGAATACTTNGGTAAATTTGTCCTGGAGAGAAAACTTATCAGACAG

Fig 1J

4073-1
>2 00005
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Fig 1K

3321-1

>RV1 00006

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3321-1 p. 2

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Fig 1 L

3216-1

>1-5 00001

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Fig 1M

4072-2

>2-2 00007

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Fig 1N

950-1

>950-1 00005

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Fig 10

950-2

>Y950-2 00002

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Fig 1P

950-3

>950-3 00001

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ggagagaagttagtctttttctcttaagtatttttcaactgataggaagcaatctgctt
tactcaaagtgtactgaattaaatgttaatctcatccagaagtaccttcacatcaacgtc
taaactagtgttcgatcaaatatctgaatactatgagctagccatgttgacacgaaaaat
taaccattgnatatgttttcacttcttttccaggaaatacctaggaatggaattgctggt
catttagtaagtgggtgtttaactttataagaagctaccagtgttttccaaagtggtggt
tcantttacattccaaaaagcattatatgagagttccagttgnacaacctcctcagcatt
tgntattgtcagacttaatgtttatg

Fig 1R

950-4

>950-4 00007

aatctaccatagtttcagtttcattgtattttcttatatatttatattgtgtactgatgt
gtgcatggattagtaatgagtactctattatttttaatgtcataattattcattatttat
ttattatttattaaaaataatatttaatattaaatattatttattacttatattatttat
tattataataatttataatatgtcacattataaaatattatttaattaaaatttaatgtc
actctattattttaatatcataaaatacatgaaaatacatttttctgtagaatcacgttt
tcctcctactgtgaataaagacataactcactaaggggaagaatcttggcccnaagtgtg
tgataaatcananaananataaaagtgtncnnaaacaaacagtaaaggtgaaaggaggca
caaatttaataaagttactccataaatcataattgacattaaatgttggaatgtaggaac
tgatttattaaccatataaatttaaaacacacatgttatcttttgacaaattgtttacct
attttagttttcaaagtgggcaaaattaacacctcaaaacatataagtgttttcagagaa
ggatcacagagtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtataatggtaatntg
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cctttttccagaagaactgggaaaacttttcatatttaagtttggaacaacagagagaac
gggaagactttggcatttagagaatgtgaatatttgtatttctcgataaagtgagaaatc
tttgtggaaaagctatggctttantcagtttcaaatctgagacccctttcttgtaggcag
atgtgctaaccanttnaccccaaaaaactttctcttctgtgcagcaatccatagcagaat
gaaaggaggattctttggatatactcaaacctaataactt

Fig 15

950-5
>950-5 00004
ctgctgagcattgtgtccctgtgagcccctctacgttattttacctctgactggaacaca
cctccatcatttttccctcacttcctgcaagttgctgtctaaaggtgactgttccacatt
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ggcctcatgaataatgagtcagtgcttcgatgtctcatatatctgtgaattctgaatgtg
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atcttaagcatttttgcctacattttactaactaaaagcatatttatcttttataataga
attgctcatttacaaaagtaaatatgtgttacaatccatatcatttattggtgtatccct
gacatatagtaggcactcaatacatatatggaatgagagaattgtgctttctctctccct
tttcgccttcccctcctcctgctttttctcctactctcacactgtctctctctctcaaac
acacacacacacacacacacacacacacacacacacacattggacaggaatcccagagat
ctgggttctggcgtgacagcatgtttttcacaaatacttcttggtttccattccttcaaa
tgtaaataaaggtagggtttagtaagatgatctttgagtttccttccaggattcagagtt
tcatcaataatttctttattcctttgctctacaaggtttctttgtgctgtggctttaatg
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attttgtattaagcaagctaaaaccagagaaagattagatttaaaacctttaaaagtga
agctaggagaggtgcctcatgtctatagtcccagctacttgggaggctggagtaggaaga
tagcttgagcccagaagttcaaggctgcag

Fig 1T

950-6
>Y950-6 00004

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aatcctttaatatctttatgacttctttccctttctccccagttcctgttttctcatgtt
caagctctgacattcaaaactaaacacctttctctaacatgttgctttaattatttaagc
attctgcctgggatattttcagttagtcatgggatttttcataaaactctcccaatatat
ctccaagtggccaggcttttcaatcactgcttccctccatgtgttttcacacacacacac
acacacacacacacactctccacttaaattgaacaggtttatttctttacacaagaattc
ttacaaacagcccggttttctccaccatatgtccactccttctctgcatagctcaatttt
gattcttacactatattttacatattcttacactctgatacgatcttgtctcttattctt
tatggctctgctctgtaattttgttgttgttgttctgaaatatagttggacatgtaactt
gtacatgacacaccttagcaaggaggcaactcatatctcagatgtaagtgaaagaagcac
tctccaggggtttcctatgggagtggtcagcacgctggcctcattggtggaatggcctag
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acag

Fig 1-U

950-8
>Y950COS8 00002
ctgcagatatgggcttcattaatt
acattgtttttcaagtctctctttctatatatatgtgtgtgtgtgtgtgtgtgtgtgt
gtgtgtgtgtgtgttgtttttgtttttgtttttgagacagaacctcactctgtctcccag
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agcctcccaagtagctgggactacagctgcag

Fig 1V

```
>63-1        00001
tcgactctagaggatcccccnaggggaagaccaagtctgggacttcagcgccagcctcgc
caccctgcttcccacaaccagatgtctcctgcgaagtagcccccctctaatgccaaaccaa
aagcagaggtcataaggactatatccccttacacacacacacacacacacacacacaccct
aacggctaacttctctggaaggtgcctcatccaagcaaagcagaactgaagatacttttta
catgcctttttccgttatttttttatccaatcagactttttcagacttcctttgaatacaa
gtatctgcaggattttnccttgtncacctgtagacactttttctcttttncaaggaaggg
tac
```

Fig 1W

					SEQ N
AG77	HHp1-A	CACCAAGTACACCAGCTC	PCR	HHp1	31
AG78	HHp1-B	ACTCACACGCAAAAAGCC	PCR	HHp1	32
AG64	HHp1/3'OLA	p-CTTCCAGAGAAAGAGCCTGT-dig	OLA	HHp1	33
AG62	HHp1/5'OLA-G	bio-TCTTTTCAGAGCCACTCACG	OLA	HHp1	34
AG63	HHp1/5'OLA-A	bio-TCTTTTCAGAGCCACTCACA	OLA	HHp1	35
AG110	HHp19-A	CTAACAATCAATAAAATACACTC	PCR	HHp19	36
AG111	HHp19-B	ATACCCAAGAAAATTCAAAAG	PCR	HHp19	37
AG143	HHp19/3'OLA	p-AGACAATTAAGAATGTGAGGT - dig	OLA	HHp19	38
AG144	HHp19/5'OLA-A	bio-ATATATCTATAATCTATATTTCTTA	OLA	HHp19	39
AG145	HHp19/5'OLA-G	bio-ATATATCTATAATCTATATTTCTTG	OLA	HHp19	40
AG165	HHp[redacted]29-A	CTTCCTCTCTTCCATATC	PCR	HHp26-29	41
AG166	HHp[redacted]29-B	CCCTCTATATTAGGTTTTC	PCR	HHp26-29	42
AG190	HHp29/3'OLA	p-TTTTAAAAAATGTTTAATCTTTGTG - dig	OLA	HHp29	43
AG191	HHp29/5'OLA-T	bio-TTGGGGATTTTATAGATTTTAT	OLA	HHp29	44
AG192	HHp29/5'OLA-G	bio-TTGGGGATTTTATAGATTTTAG	OLA	HHp29	45

Fig. 2

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06352

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12P 19/34; C07H 21/04 ; G11C 11/00, 15/00, 17/00
US CL : 435/6, 91.1, 91.2; 536/24.33; 360/50, 97
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.1, 91.2; 536/24.33, 24.31, 24.32; 360/50, 97

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN: Biosis, Embase, Medline, Scisearch, CJACS, DDFU, DrugU, Embal, NLDB, PROMT, CAPLUS, and Biotechds; EST-STS,n-geneseq, embl-new, genbank (nucleic acid sequence databases)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	Gyapay, G. et al. The 1993-1994 Genethon human genetic linkage map. Nature Genetics. June 1994, Volume 7, pages 246-339, especially 278 - see CA and GT strand primers for D6S464, D6S258, and D6S265.	15, 25 2, 4, 7-12, 17
X A	Stone, C. et al. Isolation of CA dinucleotide repeats close to D6S105; linkage disequilibrium with hemochromatosis. Human Molecular Genetics. November 1994, Vol. 3, No. 11, pages 2043-2046, especially page 2045 - see primer sequences CS-3 F and R.	15, 25 2,4, 7-12, 17
A	Pearson, W. R. et al. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. April 1988, Vol. 85, pages 2444-2448, especially 2446.	24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
25 JUNE 1996

Date of mailing of the international search report
03 SEP 1996

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Worwood, M. et al. Alleles at D6S265 and D6S105 define a haemochromatosis-specific genotype. British Journal of Haematology. 1994, Vol. 86, pages 863-866, especially page 864.	2,4,7-12, 17
A,P	Niederau, C. et al. Disease Associations: Which Factors Determine the Development of Liver Cancer in Hereditary Hemochromatosis?. Cancer Biotechnology Weekly. 11 March 1996, page 415.	20,21
A	Piperno, A. et al. Liver Damage in Italian Patients with Hereditary Hemochromatosis is Highly influenced by Hepatisis B and C Virus Infection. J. Hepatol. 1992, Vol. 16, No. 3, pages 364-368.	20,21
A,P	Rubin, R.B. et al. Iron and chronic viral hepatitis: emerging evidence for an important interaction. Digestive Diseases. July 1995, Vol. 13, No.4, pages 223-238.	20,21
A,P	Arber, N. et al. Elevated serum iron predicts poor response to interferon treatment in patients with chronic HCV infection. Digestive Diseases and Sciences. November 1995, Vol. 40, No. 11, pages 2431-2433.	20,21

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Wilbur, W.J. et al. Rapid similarity searches of nucleic acid and protein data banks. Proc. Natl. Acad. Sci. February 1983, Vol. 80, pages 726-730, especially page 728.	24
A,P	Beutler, E. et al. A Strategy for Cloning the Hereditary Hemochromatosis Gene. Blood Cells, Molecules and Diseases. November 1995, Volume 21, No. 21, pages 207-216, especially 213.	1-25
A,P	Burt, M.J. et al. A 4.5-Megabase YAC Contig and Physical Map over the Hemochromatosis Gene Region. Genomics. February 1996, Vol. 33, pages 153-158, especially page 156.	1-25
A,P	Totaro, A. et al. Hereditary Hemochromatosis: Generation of Transcription Map within a refined and Extended Map of the HLA Class I Region. Genomics. 1996, Vol. 31, pages 319 - 326, especially page 322.	1-25
A	Calandro, L.M. et al, Characterization of a recombinant that locates the hereditary hemochromatosis gene telomeric to HLA-F. Human Genetics. 1995, Vol. 96, pages 339-342, especially page 340.	1-25
A	Gasparini, P. et al. Linkage analysis of 6p21 polymorphic markers and the hereditary hemochromatosis: localization of the gene centromeric to HLA-F. Human Molecular Genetics. May 1993, Vol. 2, No. 5, pages 571-576, especially page 572.	1-25
A	Jazwinska, E.C. et al. Localization of the Hemochromatosis Gene Close to D6S105. American J. of Hum. Genetics. 1993, Vol. 53, pages 347-352, especially page 349.	1-25
A	Totaro, A. et al, New Markers and Polymorphisms in the Hereditary Hemochromatosis (HFE) Gene Region. Miami Biotechnology Short Report: Conference Proceedings Mol. Biol. Hum. Diseases. November 1994, Vol. 5, page 53.	1-25
A	Totaro, A. et al. New Polymorphisms and Markers in the HLA class I Region: relevance to hereditary hemochromatosis (HFE). Human Genetics. April 1995, Vol. 95, No. 4, pages 429-434, especially page 430.	1-25
A	Yaouang, J. et al. Anonymous Marker Loci within 400kb of HLA-A Gnerate Haplotypes in Likage Disequilibrium with the Hemochromatosis Gene (HFE). Am. J. Human Genetics. 1994, Vol. 54, pages 252-263, especially 256.	1-25

INTERNATIONAL SEARCH REPORT	International application No. PCT/US96/06352

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06352

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-23, and 25, drawn to methods, primers and kits used in the determination of the presence or absence of hereditary hemochromatosis.
Group II, claim 24, drawn to a computer-readable medium having a sequence recorded thereon.

The computer-readable medium with a recorded sequence of Group II neither produces the products of Group I nor uses the methods of Group I and therefore, is not linked by a special technical feature with Group I as to form a single inventive concept.